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PATRICIA K. HIMENES
Typed or Printed Name of Person Mailing Paper or Fee

Patricia K Himenes
Signature of Person Mailing Paper or Fee

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

James S. HUSTON, et al.

Serial No.: CON of 09/558,741 Art Unit: 1642

Filing Date: on even date Examiner: A. Harris

Title: BIOSYNTHETIC BINDING PROTEINS FOR IMMUNO-TARGETING

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to calculation of the filing fee and examination of the above-identified application, entry of the following amendments and remarks is respectfully requested.

Accompanying Documents

The following documents accompany this Amendment:

(1) Marked-up claim and specification pages, incorporating the amendments made herein; and

(2) Copy of the currently pending claims, incorporating the amendments made herein.

AMENDMENT

In the Specification:

On page 16, line 30, after "SEQ ID NOS.: 1 and 2." please insert the following:

--The CDRs of the 520C9 antibody are set forth in the Sequence Listing as amino acid residue numbers 31 through 35, 50 through 66, 99 through 104, 157 through 167, 183 through 189, and 222 through 230 in SEQ ID NOs 5 and 6.--

On page 18, line 12, after "fragments." please insert the following:

--The single chain Fv and sFv fusion proteins of this invention offer fewer cleavage sites to circulating proteolytic enzymes and thus offer greater stability. They reach their target tissue more rapidly, and are cleared more quickly from the body, which makes them ideal imaging agents for tumor detection and ideal radioimmunotherapeutic agents for tumor killing. They also have reduced non-specific binding and immunogenicity relative to murine immunoglobins.--

On page 46, line 19, after "linker." please insert the following:

--The gene contains nucleic acid sequences encoding V_H and V_L regions of the 520C9 antibody described above linked together with a double-stranded synthetic

oligonucleotide coding for a peptide with the amino acid sequence set forth in the Sequence Listing as amino acid residue numbers 116-133 in SEQ ID NOs: 5 and 6.--

In the Claims:

Please cancel claims 1-32, 34-41 and 44-49 without prejudice or disclaimer, and amend claims 33 and 42 as follows:

33. (Amended) An isolated polypeptide comprising:

an amino acid sequence comprising an ordered arrangement of three complementarity determining regions (CDRs) interposed between framework regions (FRs), said FRs derived from a human immunoglobulin, wherein the sequence of amino acids of said ordered arrangement of three CDRs has at least 70% sequence identity to the sequence of amino acids of an ordered arrangement of three CDRs selected from the group consisting of amino acid residue numbers 31-35, 50-66, 99-104 of SEQ ID NO:6; and amino acid residue numbers 157-167, 183-189, 222-230 of SEQ ID NO:6.

42. (Amended) An isolated DNA molecule comprising a coding sequence encoding a polypeptide, the polypeptide comprising:

an amino acid sequence comprising an ordered arrangement of three complementarity determining regions (CDRs) interposed between framework regions (FRs), wherein the sequence of amino acids of said ordered arrangement of three CDRs has at least 70% sequence identity to the sequence of amino acids of an ordered arrangement of three CDRs selected from the group consisting of amino acid residue numbers 31-35, 50-66, 99-104 of SEQ ID NO:6; and amino acid residue numbers 157-167, 183-189, 222-230 of SEQ ID NO:6.

Please add the following new claims:

--50. (New) The DNA molecule of claim 42, wherein said FR sequences are human immunoglobulin framework region sequences.

51. (New) The DNA molecule of claim 42, wherein the amino acid sequence has the general formula FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein FR1, FR2, FR3 and FR4 are framework regions and CDR1 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, CDR 2 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6 and CDR 3 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6; or CDR1 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, CDR2 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and CDR3 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6.

52. (New) The DNA molecule of claim 51, wherein CDR1 is the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, CDR 2 is the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6 and CDR 3 is the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6; or CDR1 is the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, CDR2 is the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and CDR3 is the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6,

and further wherein the polypeptide is capable of binding c-erbB-2.

53. (New) The DNA molecule of claim 42, wherein the coding sequence encodes a first polypeptide comprising a first amino acid sequence of the general formula FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 and a second polypeptide comprising a second amino acid sequence of the general formula FR1'-CDR1'-FR2'-CDR2'-FR3'-CDR3'-FR4', wherein FR1, FR2, FR3, FR4, FR1', FR2', FR3' and FR4' are framework regions and each of CDR1, CDR2, CDR3, CDR1', CDR2' and CDR3' is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6, respectively.

54. (New) The DNA molecule of claim 53, wherein said first and second polypeptides together are capable of forming a binding site for c-erbB-2.

55. (New) The DNA molecule of claim 54, wherein each of CDR1, CDR2, CDR3, CDR1', CDR2' and CDR3' is the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6, respectively,

and further wherein said first and second polypeptides together are capable of forming an antibody immunologically reactive with c-erbB-2.

56. (New) The DNA molecule of claim 55, wherein said first and second polypeptides together are capable of forming a humanized antibody.

57. (New) The DNA molecule of claim 56, wherein said FR sequences are human immunoglobulin framework region sequences of a human myeloma antibody.

58. (New) A recombinant vector comprising the DNA molecule of claim 42 operably linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

59. (New) A recombinant vector comprising the DNA molecule of claim 50 operably linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

60. (New) A recombinant vector comprising the DNA molecule of claim 53 operably linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

61. (New) A recombinant vector comprising the DNA molecule of claim 55 operably linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

62. (New) A recombinant vector comprising the DNA molecule of claim 56 operably linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

63. (New) A host cell comprising the recombinant vector of claim 58.

64. (New) A host cell comprising the recombinant vector of claim 59.

65. (New) A host cell comprising the recombinant vector of claim 60.

66. (New) A host cell comprising the recombinant vector of claim 61.

67. (New) A host cell comprising the recombinant vector of claim 62.

68. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 63; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

69. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 64; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

70. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 65; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

71. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 66; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

72. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 67; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

73. (New) The polypeptide of claim 33, wherein the amino acid sequence has the general formula FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein FR1, FR2, FR3 and FR4 are framework regions and CDR1 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, CDR 2 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6 and CDR 3 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6; or CDR1 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, CDR2 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and CDR3 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6.

74. (New) The polypeptide of claim 73, wherein CDR1 is the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, CDR 2 is the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6 and CDR 3 is the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6; or CDR1 is the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, CDR2 is the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and CDR3 is the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6,

and further wherein the polypeptide is capable of binding c-erbB-2.

75. (New) The polypeptide of claim 33, wherein the coding sequence encodes a first polypeptide comprising a first amino acid sequence of the general formula FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 and a second polypeptide comprising a second amino acid sequence of the general formula FR1'-CDR1'-FR2'-CDR2'-FR3'-CDR3'-FR4', wherein FR1, FR2, FR3, FR4, FR1', FR2', FR3' and FR4' are framework regions and each of CDR1, CDR2, CDR3, CDR1', CDR2' and CDR3' is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6, respectively.

76. (New) The polypeptide of claim 75, wherein said first and second polypeptides together are capable of forming a binding site for c-erbB-2.

77. (New) The polypeptide of claim 76, wherein each of CDR1, CDR2, CDR3, CDR1', CDR2' and CDR3' is the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6, respectively,

and further wherein said first and second polypeptides together are capable of forming an antibody immunologically reactive with c-erbB-2.

78. (New) The polypeptide of claim 77, wherein said first and second polypeptides together are capable of forming a humanized antibody.

79. (New) The polypeptide of claim 78, wherein said FR sequences are human immunoglobulin framework region sequences of a human myeloma antibody.--

REMARKS

Claims 1-32, 34-41 and 44-49 have been canceled without prejudice and without disclaimer. Applicants expressly reserve the right to bring the subject matter of the original claims again in a subsequent, related application.

The specification has been amended to recite subject matter incorporated by reference, as detailed below. Claims 33 and 42 have been amended and new claims 50-79 added. Thus, the claims now pending in the application are claims 33, 42, 43 and 50-79.

Basis for the amendment to the specification on page 16, at line 30, can be found on page 6, lines 29-35 of parent application USSN 07/831,967, which corresponds to

U.S. Patent No. 5,877,305 (U.S. Patent No. 5,877,305 was USSN 08/356,756, which was a continuation of USSN 07/831,967). The disclosure of USSN 07/831,967 is expressly incorporated by reference into the present application at page 1 of the specification. The reference numbers for the SEQ IDs have been changed relative to the parent application in order to correspond to the Sequence Listing in the present application. The corresponding text in U.S. Patent No. 5,877,305 was as follows: "The CDRs of the 520C9 antibody are set forth in the Sequence Listing as amino acid residue numbers 31 through 35, 50 through 66, 99 through 104, 157 through 167, 183 through 189, and 222 through 230 of SEQ ID NOs. 3 and 4." See, U.S. Patent No. 5, 877,305, col. 4, lines 3-7. SEQ ID NOs 5 and 6 of the present application correspond to SEQ ID NOs 3 and 4 of the parent application. Accordingly, the appropriate SEQ ID NOs have been included in the present amendment.

Basis for the amendment to the specification on page 18, at line 12, can be found on page 9, line 27, through page 10, line 1 of parent application USSN 07/831,967 (U.S. Patent No. 5,877,305, col. 5, lines 29-37), the disclosure of which is incorporated by reference into the present application.

Basis for the amendment to the specification on page 46, at line 19, can be found on page 34, lines 20-27 of parent application USSN 07/831,967 (U.S. Patent No. 5,877,305, col. 16, lines 58-64), the disclosure of which is incorporated by reference into the present application. The reference numbers for the SEQ IDs have been changed relative to the parent application in order to correspond to the Sequence Listing in the present application.

Claims 33 and 42 have been amended and new claims 50-79 added. Claims 50-57 recite DNA molecules which include sequences encoding 520C9 CDRs. Claims 73-79 recite polypeptides which include 520C9 CDRs. Claims 58-62 relate to recombinant vectors comprising the DNA molecules; claims 63-67 pertain to host cells comprising the recombinant vectors; and claims 68-72 relate to methods of recombinant production.

Basis for the amendments and new claims is as follows. Basis for the recitations in all the claims to CDRs for 520C9 (amino acid residue numbers 31-35, 50-66, 99-104 of SEQ ID NO:6; and amino acid residue numbers 157-167, 183-189, 222-230 of SEQ ID NO:6) can be found at least in the amendment made to the specification on page 16, at line 30. The amendment to page 16 is information incorporated from the parent application (see above).

Basis for the recitation regarding an amino acid sequence comprising an ordered arrangement of three CDRs interposed between FRs can be found throughout the specification as originally filed, for example, at the following locations: page 16, line 23, to page 17, line 1; page 29, line 15; and page 36, lines 9-23. Basis for the amendment reciting that the CDRs have sequences with the stated percent sequence identity may be found at, e.g., page 10, lines 9-21. Additional support for the recitations in the new claims can be found throughout the specification, at, for example, page 6, lines 6-7; page 6, lines 17-29; page 8, lines 24-29; page 9, lines 22-24; page 10, line 22; page 36, lines 18-19; and page 36, lines 21-23.

Basis for new claims 58-72, directed to various recombinant aspects of the invention, can be found throughout the application at, *inter alia*, page 26, lines 3-7; page 26, lines 18-22; page 37, line 14 through page 39, line 16.

Accordingly, no new matter has been entered by way of this amendment and entry thereof is respectfully requested.

Please direct all further written communications in this application to:

Joseph H. Guth, Esq.
Chiron Corporation
Intellectual Property - R440
P.O. Box 8097
Emeryville, CA 94662-8097

Respectfully submitted,

Date: 6/21/01

By: _____
Roberta L. Robins
Registration No. 33,208
Attorney for Applicants

CHIRON CORPORATION
Intellectual Property - R440
P.O. Box 8097
Emeryville, CA 94662-8097
Telephone: (510) 923-3888
Facsimile: (510) 655-3542

erbB-2-related tumor antigen" is a protein located on the surface of tumor cells, such as breast and ovarian tumor cells and which is antigenically related to the c-erbB-2 antigen. That is, the related antigen can be bound by an immunoglobulin that is capable of binding the c-erbB-2 antigen (e.g. 741F8, 520C9, and 454C11 antibodies. Related antigens also include antigens comprising an amino acid sequence that is at least 80% homologous, preferably 90% homologous, with the amino acid sequence of c-erbB-2 or an amino acid sequence encoded by a DNA that hybridizes under stringent conditions with a nucleic acid sequence encoding c-erbB-2. As used herein, stringent hybridization conditions are those set forth in Sambrook, et al., 1989, Molecular Cloning; A Laboratory Manual 2nd ed. Cold Spring Harbor Press wherein the hybridization conditions, for example, include 50% formamide, 5x Denhardt's Solution, 5xSSC, 0.1% SDS and 100 μg/ml denatured salmon sperm DNA and the washing conditions include 2xSSC, 0.1% SDS at 37°C followed by 1xSSC, 0.1% SDS at 68°C. An example of a c-erbB-2-related antigen is the receptor for the epidermal growth factor.

In one embodiment, the biosynthetic antibody binding site is a humanized hybrid molecule which includes CDRs from the mouse 741F8 antibody interposed between FRs derived from one or more human immunoglobulin molecule. The CDRs that bind to the c-erbB-2 epitope can be found in the amino acid residue numbers 31-37, 52-68, 101-110, 159-169, 185-191 and 224-233 in SEQ ID NOS.: 1 and 2. **The CDRs of the 520C9 antibody are set forth in the Sequence Listing as amino acid residue numbers 31 through 35, 50 through 66, 99 through 104, 157 through 167, 183 through 189, and 222 through 230 in SEQ ID NOs 5 and 6.** The hybrid molecule thus contains binding sites which are highly specific for the c-erbB-2 antigen or c-erbB-2 related antigens held in proper immunochemical binding conformation by human FR amino acid sequences, which are less likely to

resonance imaging. Overexpression of c-erbB-2 or related receptors on malignant cells thus allows targeting of sFv' species to the tumor cells, whether the tumor is well-localized or metastatic. In addition, internalization of an sFv-toxin fusion protein permits specific destruction of tumor cells bearing the overexpressed c-erbB-2 or related antigen.

The present invention discloses monomeric and dimeric biosynthetic constructs having enhanced properties as in vivo targeting agents when compared with intact monoclonal antibodies or their Fab fragments. **The single chain Fv and sFv fusion proteins of this invention offer fewer cleavage sites to circulating proteolytic enzymes and thus offer great stability. They reach their target tissue more rapidly, and are cleared more quickly from the body, which makes them ideal imaging agents for tumor detection and ideal radioimmunotherapeutic agents for tumor killing. They also have reduced non-specific binding and immunogenicity relative to murine immunoglobins.** The dimeric biosynthetic constructs of the invention also permit the in vivo targeting of an epitope on an antigen with greater apparent avidity, including greater tumor specificity, tumor localization and tumor retention properties than that of the Fab fragment having the same CDRs as the construct. Furthermore, the dimeric constructs also permit the in vivo targeting of an epitope on an antigen with a greater apparent avidity, including greater tumor localization and tumor retention properties, than either of the monomeric polypeptides individually.

The invention also includes methods for producing the homo- and heterodimeric biosynthetic constructs, which include the steps of designing, constructing, expressing, purifying, and refolding the monomeric sFv' polypeptide chains in vitro, followed by joining two polypeptide chains together through the crosslinking means in the C-terminal tail sequence, without relying on the tail structure to otherwise assist in dimer formation or enhance transport across a membrane. The invention also includes methods for imaging a preselected antigen in a mammal expressing the preselected antigen. The antigen may be expressed on a

into a Hpa I restriction site close to the 3' end of the 26-10 sFv gene. The resulting sFv' gene, set forth in the Sequence Listing as SEQ. ID. NOS.: 3 and 4, was then inserted into the E. coli expression vector pET-3d. This plasmid was subsequently transformed into E. coli BL21-DE (In-vitrogen, Inc.) and protein expression induced by the addition of IPTG to the culture medium.

C. 520C9 sFv .

The 520C9 sFv was generated by linking together the V_H and V_L genes, cloned from a 520C9 hybridoma cDNA library, with a serine rich linker. Briefly, the V_H and V_L genes were cloned from the 520C9 hybridoma cDNA library using probes directed toward the antibody constant (C) and joining (J) regions. Appropriate restriction sites were introduced at the ends of each gene by site-directed mutagenesis (Kunkel et al., 1985, Proc. Natl. Acad. Sci. USA 82: 488-492). The V_H and V_L genes were then ligated together with a serine rich linker. **The gene contains nucleic acid sequences encoding V_H and V_L regions of the 520C9 antibody described above linked together with a double-stranded synthetic oligonucleotide coding for a peptide with the amino acid sequence set forth in the Sequence Listing as amino acid residue numbers 116-133 in SEQ ID Nos: 5 and 6.** The resulting 520C9 sFv gene, set forth in the Sequence Listing as SEQ. ID. NOS.: 5 and 6, was transformed into the E. coli expression vector and expressed as described above and in co-pending USSN 831,967, incorporated therein by reference.

EXAMPLE 2. Renaturation, Dimerization and Purification of sFv Proteins

A. Renaturation and Purification of sFv Monomers

Protocols for renaturing sFv monomers derived from E. coli inclusion bodies are described below. In separate experiments the 7418, 26-10 and 520C9 sFv

Marked-Up Claims (CON of 09/558,741)

1-32 (Canceled)

33. (Amended) [A single-chain Fv (sFv)] An isolated polypeptide [for binding preferentially to a c-erbB-2 or a c-erbB-2-related tumor antigen, the polypeptide] comprising:

an amino acid sequence [defining at least two polypeptide domains, connected by a polypeptide linker spanning the distance between the C-terminus of one domain and the N-terminus of the other, the amino acid sequence of each said domain] comprising an ordered arrangement of three complementarity determining regions (CDRs) interposed between framework regions (FRs), said FRs derived from a human immunoglobulin, wherein the sequence of amino acids of said ordered arrangement of three [the] CDRs [and FRs of each polypeptide chain together defining a binding site immunologically reactive with said c-erbB-2 or c-erbB-2-related tumor antigen] has at least 70% sequence identity to the sequence of amino acids of an ordered arrangement of three CDRs selected from the group consisting of amino acid residue numbers 31-35, 50-66, 99-104 of SEQ ID NO:6; and amino acid residue numbers 157-167, 183-189, 222-230 of SEQ ID NO:6.

34-41 (Canceled)

42. (Amended) [A] An isolated DNA molecule comprising a coding sequence encoding [the] a polypeptide, the polypeptide comprising:

an amino acid sequence comprising an ordered arrangement of three complementarity determining regions (CDRs) interposed between framework regions (FRs), wherein the sequence of amino acids of said ordered arrangement of three CDRs has at least 70% sequence identity to the sequence of amino acids of an ordered arrangement of three CDRs selected from the group consisting of amino acid residue numbers 31-35, 50-66, 99-104 of SEQ ID NO:6; and amino acid residue numbers 157-167, 183-189, 222-230 of SEQ ID NO:6 [chain of claim 1, 2, 3 or 33].

44-49 (Canceled)

50. (New) The DNA molecule of claim 42, wherein said FR sequences are human immunoglobulin framework region sequences.

51. (New) The DNA molecule of claim 42, wherein the amino acid sequence has the general formula FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein FR1, FR2, FR3 and FR4 are framework regions and CDR1 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, CDR 2 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6 and CDR 3 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6; or CDR1 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, CDR2 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and CDR3 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6.

52. (New) The DNA molecule of claim 51, wherein CDR1 is the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, CDR 2 is the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6 and CDR 3 is the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6; or CDR1 is the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, CDR2 is the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and CDR3 is the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6,

and further wherein the polypeptide is capable of binding c-erbB-2.

53. (New) The DNA molecule of claim 42, wherein the coding sequence encodes a first polypeptide comprising a first amino acid sequence of the general formula FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 and a second polypeptide comprising a second amino acid sequence of the general formula FR1'-CDR1'-FR2'-CDR2'-FR3'-CDR3'-FR4', wherein FR1, FR2, FR3, FR4, FR1', FR2', FR3' and FR4' are framework regions and each of CDR1, CDR2, CDR3, CDR1',

CDR2' and CDR3' is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6, respectively.

54. (New) The DNA molecule of claim 53, wherein said first and second polypeptides together are capable of forming a binding site for c-erbB-2.

55. (New) The DNA molecule of claim 54, wherein each of CDR1, CDR2, CDR3, CDR1', CDR2' and CDR3' is the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6, respectively,

and further wherein said first and second polypeptides together are capable of forming an antibody immunologically reactive with c-erbB-2.

56. (New) The DNA molecule of claim 55, wherein said first and second polypeptides together are capable of forming a humanized antibody.

57. (New) The DNA molecule of claim 56, wherein said FR sequences are human immunoglobulin framework region sequences of a human myeloma antibody.

58. (New) A recombinant vector comprising the DNA molecule of claim 42 operably linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

59. (New) A recombinant vector comprising the DNA molecule of claim 50 operably linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

60. (New) A recombinant vector comprising the DNA molecule of claim 53 operably linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

61. (New) A recombinant vector comprising the DNA molecule of claim 55 operably linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

62. (New) A recombinant vector comprising the DNA molecule of claim 56 operably linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

63. (New) A host cell comprising the recombinant vector of claim 58.

64. (New) A host cell comprising the recombinant vector of claim 59.

65. (New) A host cell comprising the recombinant vector of claim 60.

66. (New) A host cell comprising the recombinant vector of claim 61.

67. (New) A host cell comprising the recombinant vector of claim 62.

68. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 63; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

69. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 64; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

70. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 65; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

71. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 66; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

72. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 67; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

73. (New) The polypeptide of claim 33, wherein the amino acid sequence has the general formula FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein FR1, FR2, FR3 and FR4 are framework regions and CDR1 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, CDR 2 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6 and CDR 3 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6; or CDR1 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, CDR2 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and CDR3 is a sequence of amino

acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6.

74. (New) The polypeptide of claim 73, wherein CDR1 is the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, CDR 2 is the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6 and CDR 3 is the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6; or CDR1 is the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, CDR2 is the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and CDR3 is the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6,

and further wherein the polypeptide is capable of binding c-erbB-2.

75. (New) The polypeptide of claim 33, wherein the coding sequence encodes a first polypeptide comprising a first amino acid sequence of the general formula FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 and a second polypeptide comprising a second amino acid sequence of the general formula FR1'-CDR1'-FR2'-CDR2'-FR3'-CDR3'-FR4', wherein FR1, FR2, FR3, FR4, FR1', FR2', FR3' and FR4' are framework regions and each of CDR1, CDR2, CDR3, CDR1', CDR2' and CDR3' is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6, respectively.

76. (New) The polypeptide of claim 75, wherein said first and second polypeptides together are capable of forming a binding site for c-erbB-2.

77. (New) The polypeptide of claim 76, wherein each of CDR1, CDR2, CDR3, CDR1', CDR2' and CDR3' is the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6, the

sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6, respectively,

and further wherein said first and second polypeptides together are capable of forming an antibody immunologically reactive with c-erbB-2.

78. (New) The polypeptide of claim 77, wherein said first and second polypeptides together are capable of forming a humanized antibody.

79. (New) The polypeptide of claim 78, wherein said FR sequences are human immunoglobulin framework region sequences of a human myeloma antibody.

Currently Pending Claims (CON of 09/558,741)

33. (Amended) An isolated polypeptide comprising:

an amino acid sequence comprising an ordered arrangement of three complementarity determining regions (CDRs) interposed between framework regions (FRs), said FRs derived from a human immunoglobulin, wherein the sequence of amino acids of said ordered arrangement of three CDRs has at least 70% sequence identity to the sequence of amino acids of an ordered arrangement of three CDRs selected from the group consisting of amino acid residue numbers 31-35, 50-66, 99-104 of SEQ ID NO:6; and amino acid residue numbers 157-167, 183-189, 222-230 of SEQ ID NO:6.

42. (Amended) An isolated DNA molecule comprising a coding sequence encoding a polypeptide, the polypeptide comprising:

an amino acid sequence comprising an ordered arrangement of three complementarity determining regions (CDRs) interposed between framework regions (FRs), wherein the sequence of amino acids of said ordered arrangement of three CDRs has at least 70% sequence identity to the sequence of amino acids of an ordered arrangement of three CDRs selected from the group consisting of amino acid residue numbers 31-35, 50-66, 99-104 of SEQ ID NO:6; and amino acid residue numbers 157-167, 183-189, 222-230 of SEQ ID NO:6.

43. A host cell transfected with a DNA of claim 42.

50. (New) The DNA molecule of claim 42, wherein said FR sequences are human immunoglobulin framework region sequences.

51. (New) The DNA molecule of claim 42, wherein the amino acid sequence has the general formula FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein FR1, FR2, FR3 and FR4 are framework regions and CDR1 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, CDR 2 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6 and CDR 3 is a sequence of amino acids

with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6; or CDR1 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, CDR2 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and CDR3 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6.

52. (New) The DNA molecule of claim 51, wherein CDR1 is the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, CDR 2 is the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6 and CDR 3 is the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6; or CDR1 is the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, CDR2 is the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and CDR3 is the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6,

and further wherein the polypeptide is capable of binding c-erbB-2.

53. (New) The DNA molecule of claim 42, wherein the coding sequence encodes a first polypeptide comprising a first amino acid sequence of the general formula FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 and a second polypeptide comprising a second amino acid sequence of the general formula FR1'-CDR1'-FR2'-CDR2'-FR3'-CDR3'-FR4', wherein FR1, FR2, FR3, FR4, FR1', FR2', FR3' and FR4' are framework regions and each of CDR1, CDR2, CDR3, CDR1', CDR2' and CDR3' is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6, respectively.

54. (New) The DNA molecule of claim 53, wherein said first and second polypeptides

together are capable of forming a binding site for c-erbB-2.

55. (New) The DNA molecule of claim 54, wherein each of CDR1, CDR2, CDR3, CDR1', CDR2' and CDR3' is the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6, respectively,

and further wherein said first and second polypeptides together are capable of forming an antibody immunologically reactive with c-erbB-2.

56. (New) The DNA molecule of claim 55, wherein said first and second polypeptides together are capable of forming a humanized antibody.

57. (New) The DNA molecule of claim 56, wherein said FR sequences are human immunoglobulin framework region sequences of a human myeloma antibody.

58. (New) A recombinant vector comprising the DNA molecule of claim 42 operably linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

59. (New) A recombinant vector comprising the DNA molecule of claim 50 operably linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

60. (New) A recombinant vector comprising the DNA molecule of claim 53 operably linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

61. (New) A recombinant vector comprising the DNA molecule of claim 55 operably

linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

62. (New) A recombinant vector comprising the DNA molecule of claim 56 operably linked to control elements, whereby the coding sequence encoding said polypeptide can be transcribed and translated in a host cell.

63. (New) A host cell comprising the recombinant vector of claim 58.

64. (New) A host cell comprising the recombinant vector of claim 59.

65. (New) A host cell comprising the recombinant vector of claim 60.

66. (New) A host cell comprising the recombinant vector of claim 61.

67. (New) A host cell comprising the recombinant vector of claim 62.

68. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 63; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

69. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 64; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

70. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 65; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

71. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 66; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

72. (New) A method of producing a recombinant polypeptide comprising:

(a) providing a population of host cells according to claim 67; and

(b) culturing said population of cells under conditions whereby the polypeptide encoded by the coding sequence present in said recombinant vector is expressed.

73. (New) The polypeptide of claim 33, wherein the amino acid sequence has the general formula FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein FR1, FR2, FR3 and FR4 are framework regions and CDR1 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, CDR 2 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6 and CDR 3 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6; or CDR1 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, CDR2 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and CDR3 is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6.

74. (New) The polypeptide of claim 73, wherein CDR1 is the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, CDR 2 is the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6 and CDR 3 is the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6; or CDR1 is the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, CDR2 is the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and CDR3 is the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6,

and further wherein the polypeptide is capable of binding c-erbB-2.

75. (New) The polypeptide of claim 33, wherein the coding sequence encodes a first polypeptide comprising a first amino acid sequence of the general formula FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 and a second polypeptide comprising a second amino acid sequence of the general formula FR1'-CDR1'-FR2'-CDR2'-FR3'-CDR3'-FR4', wherein FR1, FR2, FR3, FR4, FR1', FR2', FR3' and FR4' are framework regions and each of CDR1, CDR2, CDR3, CDR1', CDR2' and CDR3' is a sequence of amino acids with at least 90% sequence identity to the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6, respectively.

76. (New) The polypeptide of claim 75, wherein said first and second polypeptides together are capable of forming a binding site for c-erbB-2.

77. (New) The polypeptide of claim 76, wherein each of CDR1, CDR2, CDR3, CDR1', CDR2' and CDR3' is the sequence of amino acids found at amino acid positions 31-35 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 50-66 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 99-104 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 157-167 of SEQ ID NO:6, the sequence of amino acids found at amino acid positions 183-189 of SEQ ID NO:6 and the sequence of amino acids found at amino acid positions 222-230 of SEQ ID NO:6, respectively,

and further wherein said first and second polypeptides together are capable of forming an antibody immunologically reactive with c-erbB-2.

78. (New) The polypeptide of claim 77, wherein said first and second polypeptides together are capable of forming a humanized antibody.

79. (New) The polypeptide of claim 78, wherein said FR sequences are human immunoglobulin framework region sequences of a human myeloma antibody.